Figures and figure supplements

Inhibitor of ppGalNAc-T3-mediated O-glycosylation blocks cancer cell invasiveness and lowers FGF23 levels

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Figure 1. Screen for modulators of ppGalNAc-T2/T3. (A) Diagram showing sensor design and the linker sequences used. O-glycosylation of the linker masks the furin site but if an inhibitor blocks the ppGalNAc-transferase then furin cleaves the linker releasing the blocking domain (BD) allowing

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fluorescent activating protein (FAP) dimerization and dye activation. Linker furin sites are underlined and sites of glycosylation or mutation are in bold. (B) HEK cell lines with or without ppGalNAc-T2 or T3 stably expressing the WT or Δglycan T2 or T3 sensor constructs (see linkers in A) were imaged in the presence of 110 nM of the dye MG11p (MG) to detect GFP or MG. Bar = 20 μm. (C) Schematic showing cell plating, drug treatment, cell release, fluorescence measurement and parallel analysis using both T2 and T3 sensors. Hits that activate both may be pan-specific or act on off-target pathways common to both sensors whereas sensor specific hits are likely acting directly on the corresponding ppGalNAc-transferase. (D) The plot shows Q values \( Q = (R - R_{\text{Neg}}) / \text{SD}_{\text{Neg}} \) for each compound (treatment at 10 μM for 6 hr) using the average of duplicate MG/GFP ratios for the compound \( R \), the vehicle-only control \( R_{\text{Neg}} \), and the standard deviation of the vehicle-only controls \( \text{SD}_{\text{Neg}} \). The cut-off values of +3 and −2.5 are indicated (*). Also indicated are the values for the positive controls (T2Δglycan and T3Δglycan) and the structure of the indicated T3-specific hit (inset). (E) Values (% enzyme activity relative to vehicle-only controls) in the in vitro assay using purified ppGalNAc-T2 or T3 as a secondary screen are shown for 20 hits from the primary screen. Compounds were present at 50 μM. Compound 1614 is T3inh-1.

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The following source data is available for figure 1:

**Source data 1.** Primary screen data for HEK cells expressing T2 or T3 sensors.

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**Source data 2.** Secondary screen data (in vitro enzyme assays).

DOI: 10.7554/eLife.24051.005
Figure 1—figure supplement 1. Cell growth at various T3Inh-1 exposures. Identical numbers of HEK cells were plated and grown in the continuous presence of the indicated concentrations of T3Inh-1 and then at 24, 48, or 72 hr they were released and counted. Averages are shown normalized using the untreated sample at 72 hr (n = 3 ± SEM).

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The following source data is available for figure 1:

Figure supplement 1—Source data 1. Cell counts at differing time points and T3Inh-1 concentrations.

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**Figure 1—figure supplement 2.** General N- and O-glycosylation are unaffected. (A, C, E, G) Representative fluorescent emission spectra from 510–550 nm of lysates obtained from HeLa cells treated with T3Inh-1 for 24 hr at 0, 10, or 20 µM. The cells were stained with the indicated lectin for 30 min just prior to analysis. (B, D, F, H) Quantified average staining values for the indicated lectins (at 520 nm emission) and T3Inh-1 treatments (n = 3 ± SEM).

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The following source data is available for figure 1:

**Figure supplement 2—Source data 1.** Fluorescent lectin staining of cells at differing T3Inh-1 concentrations.

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**Figure 1—figure supplement 3.** ppGalNAc-transferase levels are unaffected. (A) Representative images of untreated or T3Inh-1 treated (6 hr, 10 μM) HeLa cells after fixation and staining with antibodies against the indicated ppGalNAc-transferase and the Golgi marker GPP130. Bar = 10 μm.

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Figure 2. T3Inh-1 is a direct mixed-mode inhibitor of ppGalNAc-T3. (A) Comparison of T2 and T3 sensor activation at the indicated concentrations of T3Inh-1 (n = 3 ± SEM). MG/GFP ratio was determined for 20,000 cells by FACS and average value is plotted as percent of the positive control (i.e. the glycan version of each sensor). (B) Comparison of effect of the indicated concentrations of T3Inh-1 on in vitro glycosylation mediated by purified ppGalNAc-T2, ppGalNAc-T3, or ppGalNAc-T6. Values are averages expressed as percentage of the control ‘vehicle-only’ reactions (n = 6 ± SEM for ppGalNAc-T3, n = 3 ± SEM for others). (C–D) The in vitro assay was carried out in the presence of 0, 7.5, or 15 μM T3Inh-1 at the indicated concentrations of peptide or UDP-GalNAc substrate. Values are averages expressed as percent of the control reactions with no inhibitor and saturating substrates (n = 3 ± SEM). (E) Representative fluorescence spectra are shown for T3Inh-1 alone or for purified ppGalNAc-T3 in the presence of the indicated concentrations of T3Inh-1. Note dose-dependent quenching of tryptophan fluorescence indicating direct binding. (F) Fluorescence quenching was quantified at each concentration using the peak value at 324 nm (n = 3 ± SEM). Note that all graphs have error bars but some are too small to be apparent.

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The following source data is available for figure 1:

Source data 1. Panel A: sensor signals versus T3Inh-1 concentration.
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Source data 2. Panel B: enzyme activity versus T3Inh-1 concentration.
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Source data 3. Panel C: inhibitor effect versus peptide concentration.

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